THE USE OF VERTEBRAL RINGS OF THE BROWN RAY *RAJA MIRALETUS* (LINNAEUS, 1758) OFF EGYPTIAN MEDITERRANEAN COAST FOR ESTIMATION OF AGE AND GROWTH

by

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ABSTRACT. - A simple silver nitrate technique was used to stain whole vertebrae from 547 brown rays, *Raja miraletus* caught by trawling off the Mediterranean coast of Alexandria between June 1990 and July 1991. From the validation techniques the analysis of marginal increments was selected. A single annulus forms each year on the vertebrae. The annual nature of the rings was demonstrated by correlation with length-frequency analysis for the years 1988-1991. Counts of growth rings from vertebral centra stained with silver nitrate were employed to back calculate length at age for the size range 18-70 cm TL. The growth of *R. miraletus* was described by a von Bertalanffy growth curve with parameters $k = 0.19$, $t_0 = -0.50$ and $L_\infty = 87.87$ for males and $k = 0.17$, $t_0 = -0.25$ and $L_\infty = 91.92$ for females. Low value of growth rate and moderate life span are characteristic of this species.

RÉSUMÉ. - Une technique simple au nitrate d'argent a été utilisée pour colorer les anneaux calcifiés des vertèbres entières chez 547 individus de la raie miroir, *Raja miraletus*. Ces poissons ont été pêchés par chalutage en Méditerranée devant les côtes d'Alexandrie de juin 1990 à juillet 1991. Parmi les diverses techniques de validation disponibles, l'analyse de l'accroissement marginal a été sélectionnée, technique suivant laquelle chaque anneau représente une année. La nature annuelle des anneaux a été montrée par l'analyse des fréquences de longueur pour la période de 1988 à 1991. Pour la totalité des individus de 18 à 70 cm de longueur totale, un rétrocalcul de la longueur aux différents âges a été établi à partir du compte des anneaux de croissance colorés par le nitrate d'argent. La croissance de la raie miroir a été décrite par la courbe de von Bertalanffy en utilisant les paramètres $k = 0.19$, $t_0 = -0.50$ et $L_\infty = 87.87$ pour les mâles et $k = 0.17$, $t_0 = -0.25$ et $L_\infty = 91.92$ pour les femelles. Les faibles taux de croissance et une durée de vie moyenne caractérisent cette espèce.

Key-words. - Rajidae, *Raja miraletus*, MED, Egypt, Vertebral rings, Age estimation, Growth.

A variety of skeletal structures have been used to determine age in elasmobranchs. Kaganovskaia (1933) attempted to estimate the age of the spiny dog fish, *Squalus acanthias*, using dorsal spines. Since the vertebral centrum is the only other structure known to reflect cyclic marks in elasmobranchs, whole centra have been used for age determination (Daiber, 1960; Taylor and Holden, 1964; Stevens, 1975; Cailliet *et al.*, 1983b; Allam, 1989; Cailliet, 1990; Cailliet and Tanaka, 1990). Internal surfaces of cut centra have been polished and evaluated (Lawler, 1976; Gruber and Stout, 1983; Bougis, 1989), or prepared histologically (Ishiyama, 1951; Du Buit, 1977; Casey *et al.*, 1985). Tetracycline has recently been used as a marker to validate the annual zone formation in vertebral centra (Holden and Vince, 1973; Ryland and Ajayi, 1984; Smith, 1984; Tanaka, 1990).

Moreover, length-frequency analysis has been used by several authors for age determination of elasmobranchs (Olsen, 1954; Fitz and Daiber, 1963; Richards

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et al., 1963; Sage *et al.*, 1972; Cailliet *et al.*, 1983a). Sometimes, this kind of analysis is combined with tag-recapture studies (Steven, 1947; Holden, 1974; Holden and Vince, 1973).

Vertebrae have been used for ageing rays (Ishiyama, 1951; Daiber, 1960; Richards *et al.*, 1963), with varying degrees of success. Holden and Vince (1973), Du Buit (1977) Ryland and Ajayi (1984) and Smith and Merriner (1987) showed that one opaque and one hyaline ring were deposited annually. In recent years, length-frequencies have been also used for ageing rays (Brander and Palmer, 1985; Hussein, 1985; Du Buit and Maheux, 1986).

The present study addresses age estimation and measurement of growth rate of the brown ray, *Raja miraletus* based on vertebral rings stained with silver nitrate. The results obtained were correlated with length frequency analysis.

MATERIAL AND METHODS

Our main source of brown ray, *Raja miraletus* for obtaining vertebral samples and length-frequency measurements came from the commercial trawlers operating along the Egyptian Mediterranean coast off Alexandria. For vertebral examination specimens of *Raja miraletus* were collected monthly from June 1990 to July 1991. Among the total of 547 specimens (318 males and 229 females) collected a wide range of size (11.2-71.7 cm TL) was sampled. Total length (TL) to the nearest cm and total weight (TW) to the nearest g of each fish were recorded.

The vertebrae used for age determination were removed from the branchial region adjacent to the fifth gill arch. The first 10 vertebrae were removed through a short ventral incision. Individual vertebrae were then separated and trimmed to remove excess cartilage and muscle tissue, and the connective tissue was removed from the surface of each centrum. The vertebrae were air-dried for five days.

Several different techniques were tried to reveal the rings before a specific ageing procedure was adopted. The most satisfactory technique for revealing growth rings on the vertebrae was found to be that of Stevens (1975) with some modifications. In this method, 1% silver nitrate was used for staining the vertebrae. After drying vertebrae were washed several times with distilled water, they were then transferred into 1% silver nitrate under light. This treatment was stopped as soon as the rings became visible. The stained vertebrae were cleared by immersion for 3 to 10 hr in ethylene glycol or xylene, after which the centra were viewed in xylene by reflected light over a dark background. After counting the number of rings, the distance from the centre of the centrum to the origin of the dark (opaque) rings was measured using an ocular micrometer. The centrum radius was determined as the distance from the centre to the margin of the vertebrae. The analysis of marginal increments was used according to the validation techniques described by Wootton (1990). The measurements of the vertebrae were made from the centre to the anterior margin and to the last annual growth mark. The difference between these two measurements represented the marginal vertebral increment since the last annulus was formed. Only the most abundant age groups available throughout the year-groups (I to III) were used.

Vertebral reading was back-calculated by the Dahl-Lea direct proportion method (Lagler, 1956) using the following equation

$$TLn = (TLc/VRc)(VRn)$$

where TLn = calculated total length at mark n, TLc = total length at capture, VRc = vertebral radius at capture and VRn = vertebral radius to mark n (i.e., distance to each yearly growth mark).

This method does not include a correction factor to account for the growth of the young before the vertebrae were formed. However, the author believes that the use of this method was justified because larval and embryonic growth is more

rapid than adult growth and constitutes a different growth stanza (Springer, 1960; Wootton, 1990).

Growth and performance results were subjected to one way analysis of variance. Multiple comparisons were used to compare means at the $P = 0.05$ significance level using an orthogonal polynomial procedure as described by Gill (1981).

Growth was modelled with the von Bertalanffy growth formula (VBGF) fitted to back-calculated lengths for ages 1-7. Von Bertalanffy parameters were determined using the method of Tomlinson and Abramson (1971).

The life span (t_{max}) was estimated after Taylor's concept (Taylor, 1962) and refers to the age of fish at which they attained 95% of their maximum length.

Length-frequency analysis

The monthly sample sizes were too small to consistently demonstrate polymodality and peak progression in length-frequency analysis. Consequently, data were discretely grouped combining October samples for the years (1988-1991) according to Pratt and Casey (1983). The total length of 966 specimens of *Raja miraletus* were measured to the nearest centimetre. The length-frequency distribution were divided by inspection into age classes. Size groups presumed to represent age classes were identified according to Brander and Palmer (1985) among the length-frequency data.

RESULTS

Vertebral examination

The vertebrae of *Raja miraletus* showed either opaque or transparent zones at the centrum centre. The alternating concentric pattern of broad yellow and diffuse dark brown zones on the vertebrae was easily distinguishable, particularly the first two-three rings, after staining with silver nitrate, and examination using reflected light on a dark background (Fig. 1). The marginal rings were less easily distinguished particularly in older fish (V-VII) as overstaining occasionally occurred at the centrum edge. Preliminary measurements on the vertebrae showed that the centrum radii of the two faces were not significantly different ($P > 0.05$). Both faces also showed identical ring counts. The criteria to define an annual growth mark on the vertebrae of *R. miraletus* required that the mark must occupy a distinct narrow opaque zone (dark brown) relative to wider adjacent translucent zones and could be traced around the whole vertebrae. Other marks were considered to be either juvenile or secondary (false) marks. The juvenile mark was defined as the first indistinct opaque mark close to the centrum centre (Fig. 1 a,b). The mean back calculated length for *R. miraletus* at the formation of this ring was about 12.5 cm TL. About 64% of the vertebrae examined possessed juvenile marks. Secondary (false) marks were identified and most frequently observed on the vertebrae of mature females. These marks were most often revealed as thin incomplete opaque marks (faint brown) inside the transparent zones (Fig. 1b) and rarely as continuous rings.

Time of ring formation

When vital labeling associated with tag/recapture techniques is unworkable to determine the time of vertebral ring formation, the mean monthly marginal vertebral increments of the most abundant age groups available throughout the year groups (I to III) were recorded. The marginal vertebral increment should be widest just before and narrowest just after the formation of the last annulus. As the growing season progressed, the location of the last annulus becomes more distinguished from the edge of the vertebrae (Wootton, 1990). Thus the marginal

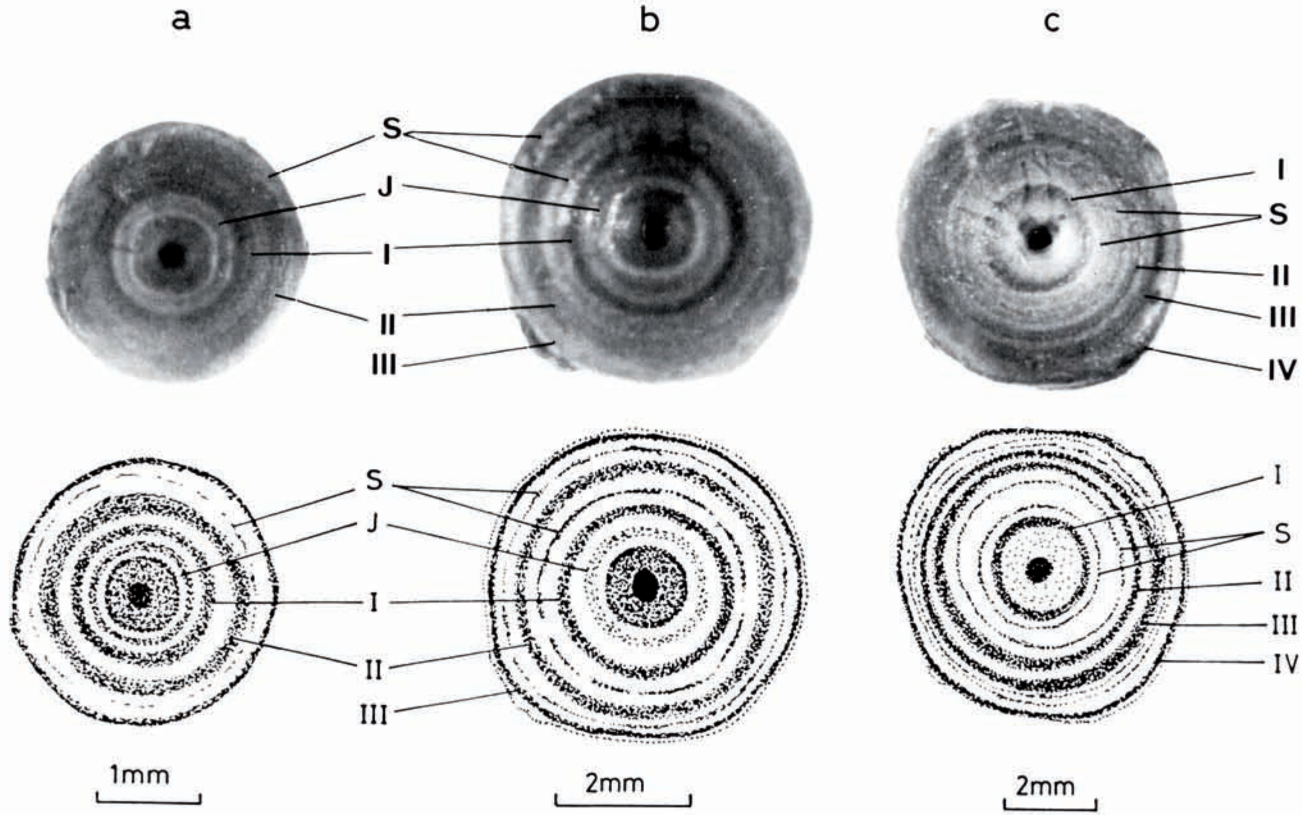

Fig. 1. - Vertebral centre of *Raja miraletus* stained with silver nitrate; a) Female, 35.7 cm TL, age 2 + years; b) Female, 46.9 cm TL, age 3 years; c) Male, 47.8 cm TL, age 4 years. J: juvenile ring; S: spawing mark.

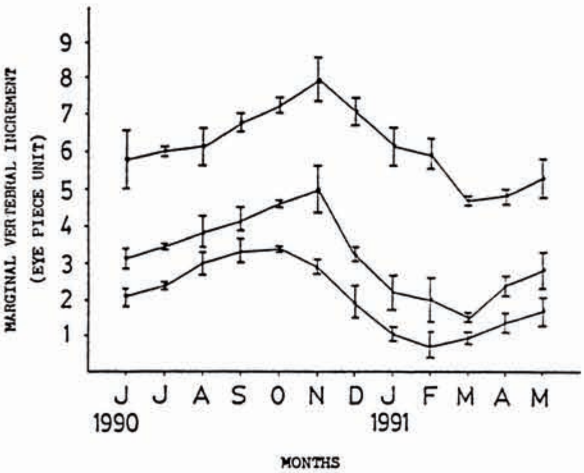

Fig. 2. - Mean monthly vertebral increment (mean + SE) of *Raja miraletus* for age groups I, II and III.

vertebral increment analysis of *Raja miraletus* (Fig. 2) indicated that a single ring (annulus) was completed each year between winter and spring with a mid point in March (where the marginal vertebral increment was minimum).

Growth

The total length/vertebral radius relationship in both sexes was best fit by a linear correlation (Fig. 3). The following equations were found to represent this relationship

$TL = 1.3736 + 11.055\ VR$ $(r^2 = 0.895)$ for females

$TL = -4.4856 + 13.3390\ VR$ $(r^2 = 0.971)$ for males

However, covariance analysis showed significant differences between sexes $(F_{0.05,\,291} = 13.11)$. Therefore, growth was estimated separately for each sex.

Back-calculated size at age of males was generally smaller than that of females (Table I; Fig. 4a). The growth did not differ statistically between sexes for age I and II individuals ($P > 0.05$), however, the difference became larger for older specimens and was significantly different ($P < 0.05$). This difference was associated with the earlier age of maturity (30-34 cm) of males when compared with females (36-40 cm).

Growth models were fitted to the back-calculated vertebral data using von Bertalanffy growth formula (VBGF). The VBGF applied for *Raja miraletus* was expressed by the following equations:

$l_t = 91.92\ [1 - e^{-0.2473(t + 0.1721)}]$ for females

$l_t = 87.87\ [1 - e^{-0.5026(t + 0.1930)}]$ for males

$l_t = 90.99\ [1 - e^{-0.3624(t + 0.1788)}]$ for combined sexes

where maximum length is given in cm.

It is clear from figure 4(a,b) that back-calculated growth curves are typical of asymptotic growth.

The estimated maximum age (t_{max}) of *R. miraletus* after Taylor's concept (Taylor, 1962) was 17.2 years for females and 15 years for males. Taken with the value of growth coefficient "k" (0.172 for females and 0.193 for males), it is apparent that *Raja miraletus* is a moderate lived, slow growing species.

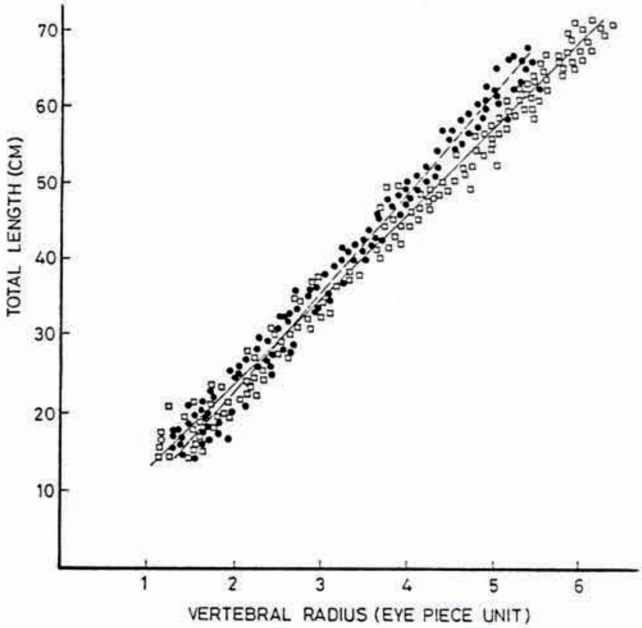

Fig. 3. - Relationship between vertebral radius and total length of *Raja miraletus* (closed circle for males and open square for females).

Table I. - Back-calculated mean length for the entire population of *R. miraletus* and comparison of back-calculated length of males and females.

	Mean back-calculated length (cm)						
	I	II	III	IV	V	VI	VII
Females							
Number of fish	25	78	58	34	18	8	8
Mean length/age	19.96	32.01	43.21	52.12	59.24	65.14	69.23
Standard deviation	5.6	2.4	3.6	4.1	5.2	8.6	6.1
Annual increment	19.90	12.05	11.20	8.91	7.12	5.90	4.09
Males							
Number of fish	40	102	86	60	14	12	4
Mean length/age	20.06	31.88	39.46	46.81	53.14	59.18	64.14
Standard deviation	3.2	6.9	8.4	3.2	4.6	2.4	9.5
Annual increment	20.06	11.82	7.58	7.35	6.33	6.04	4.96
Sex combined							
Number of fish	65	180	144	94	32	20	12
Mean length/age	20.01	31.95	41.34	49.47	56.19	62.16	66.69
Standard deviation	3.1	1.6	2.8	3.4	8.9	7.2	8.1
Annual increment	20.01	11.64	9.39	8.13	6.72	5.97	4.53

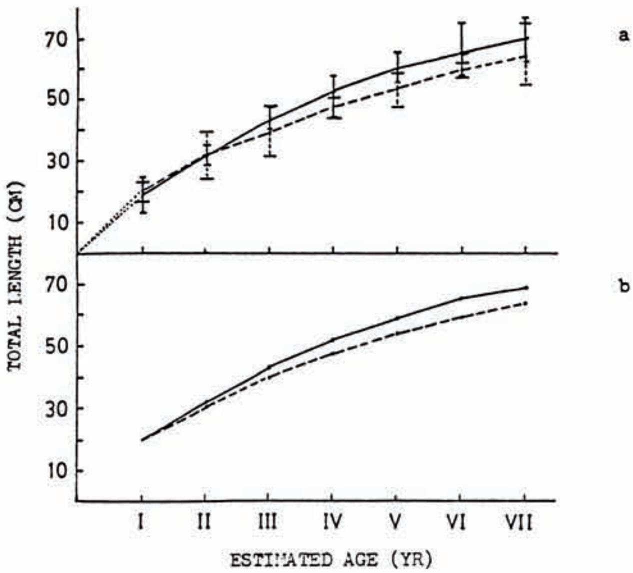

Fig. 4. - Estimated growth of *Raja miraletus* from (a) back-calculations of rings in the vertebrae (mean − SE), and (b) a von Bertalanffy growth curve for back-calculated data (full line for females and dotted line for males).

Length-frequency

Length-frequency data provide the clearest polymodal distribution when the commercial catch of *R. miraletus* was grouped by sex combined for October of years 1988-1991 (Fig. 5). The length range for each age, mean length and the number of fish in each age group is given in Table II. The estimated mean length from the length-frequency analysis approximates to size estimates from vertebrae for younger ages (I-IV) (compare results for combined sexes in Table II and Table I). Numbers in the larger size classes were insufficient to clearly demonstrate modal progression in the analysis of length data. The mean length for fish at first peak was about 12 cm (8-16 cm TL). This length is nearly equal to the back-calculated length at the formation of juvenile ring (12.52 cm TL). Thus the first peak in length frequency analysis of *Raja miraletus* can be considered as the juvenile 0 age group.

DISCUSSION

Vertebral examination

Several methods have been developed for age determination of elasmobranchs, either directly by tagging, or indirectly by length-frequency analysis or reading the growth annuli in vertebrae (Casey *et al.*, 1985). Cartilaginous vertebrae are difficult to be read directly. Therefore, some staining techniques are usually applied to assist the estimation of age by counting the concentric bands formed on vertebrae (Stevens, 1975; Pratt and Casey, 1983; Allam, 1989; Cailliet and Tanaka, 1990). The present study demonstrated that two growth marks; opaque and hyaline were deposited annually on the vertebrae of the brown ray, *Raja miraletus*, and these were most readily observed when stained with silver nitrate and viewed by reflected light on a dark back-ground. Several authors have

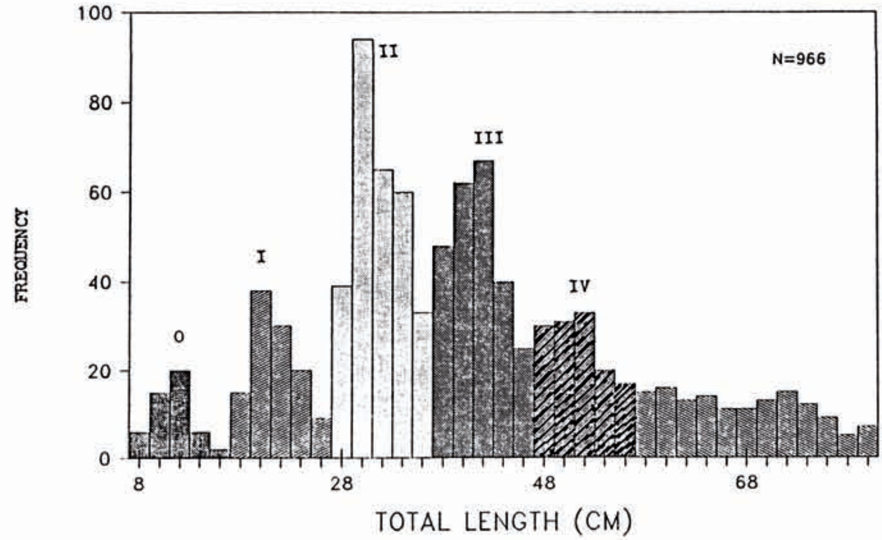

Fig. 5. - Length-frequency distributions of the brown ray (*Raja miraletus*) examined from the commercial catch off Alexandria (1988-1991).

Table II. - Length range, mean length and number derived subjectively from length-frequency distribution of *R. miraletus*.

Age group	Length range (cm)	Mean length (cm)	Number of fish
0	8-16	12.1	49
I	17-26	21.5	112
II	27-36	31.7	291
III	37-46	41.4	242
IV	47-56	51.4	131

demonstrated ring formation on the vertebrae of various *Raja* species and reported the increase in their number with fish size, (Daiber, 1960; Richard *et al.*, 1963; Taylor and Holden, 1964; Holden and Vince, 1973; Ryland and Ajayi, 1984; Smith and Merriner, 1987).

In the present study, the marginal rings were the most difficult to interpret particularly when examining the vertebrae of the larger and/or older individuals (VI-VII) of *R. miraletus* because overstaining occasionally occurs at the edges. Similar difficulties have been reported by Holden and Vince (1973) for *Raja clavata* and Ryland and Ajayi (1984) in *R. clavata*, *R. montagui* and *R. microocellata*. These authors concluded that this resulted from the presence of more connective tissue on the edge of vertebrae especially among older individuals.

Another possible source of error in analysing the growth structures in vertebrae is in distinguishing between the juvenile mark and the first annual ring. The mean back-calculated length for *R. miraletus* at the formation of juvenile mark was about 12.5 cm TL, which is slightly above the mean length of the young when they hatch (10.4 cm TL). The juvenile mark may be due to change early

feeding habits or upon transition to free feeding after the absorption of the larval yolk sac (Wootton, 1990). Abdel-Aziz (1986) and Hussein (1987) mentioned that juveniles of *Raja miraletus* fed upon small benthic or nektobenthic crustacea, while adults fed mainly upon fish.

Time of ring formation

In the present study, it was possible to detect the annual nature of vertebral rings by means of validation methods such as marginal increment and modal length progression analysis.

The marginal increment analysis method indicated that a single annulus was formed each year on the vertebrae of *R. miraletus*, between winter and spring with a mid point in March. However, Holden and Vince (1973), showed that changes in the deposition from opaque to hyaline zones usually occurs in November in *Raja clavata* from the southern North Sea. They also reported that this transition may be spread over several months and the opaque zone extended from July to December. Furthermore, Ryland and Ajayi (1984) showed that a single annulus was formed each year for three *Raja* species in Carmarthern Bay, British Isles, between December and April, with a mid point in February.

Growth

The total length-vertebral radius relationship for *R. miraletus* was found to be linear. Significant differences found in this relationship between both sexes has been previously reported (Steven, 1947; Holden, 1972; Du Buit, 1977). Conversely, Ryland and Ajayi (1984) derived a curvilinear relationship between vertebral radius and total length of *Raja clavata*, *R. microocellata* and *R. montagui* from Carmarthern Bay in the British Isles.

The back-calculation technique has normally been applied to vertebrae measurements. Growth was relatively rapid during the first year, dropped sharply in the second year and continued to decline slowly afterwards (Fig. 4a). The most likely factor responsible for the decline in growth rate with age was sexual maturation which often causes a discontinuity in growth among fish (Lagler, 1956). The smaller back-calculated size at age of males than females agreed with previous publications about the closely related species *R. clavata* (Holden, 1972; Holden and Vince, 1973) and *R. montagui* (Holden, 1972).

In male elasmobranchs, the value of the asymptotic total length (L_∞) obtained from von Bertalanffy's growth equation is usually only 0.75 that for females, and the growth coefficient (k) is twice that for females (Pauly, 1979). Holden (1972) mentioned that the ratio of L_∞ and k estimated from tagging data for both sexes of *Raja clavata* were similar to that observed for the majority of elasmobranchs. In *R. brachyura*, the reverse occurred while the values of both parameters for each sex of *R. montagui* were similar (Holden, 1972). The present results for *R. miraletus* showed that only small differences were found; the value of L_∞ for males was 0.96 of that estimated for females and the value of k was 1.12 of that calculated for females.

Rather high negative values of t_0 (Table III) are characteristic of elasmobranchs (Pauly, 1979). Holden (1972) speculated that t_0 corresponds closely to the hatching period of the eggs (t_b) in *R. clavata* and *R. montagui*. Pratt and Casey (1983) mentioned that t_0 corresponds to the gestation period in sharks. The estimated t_0 for females (-0.25 year) of *R. miraletus* in the present study does not correspond with that for t_b in contrast with males where the mean value of t_0 (-0.5 year) corresponds closely to t_b which extends for 4-5 months (Clark, 1922).

The estimated maximum age (t_{max}) for *R. miraletus* was 17.2 years for females and 15 years for males. Taken with the value of growth coefficient "k" (0.17 for females and 0.19 for males), it is apparent that *R. miraletus* is a slow growing species with a moderate life span. Table III reviews the von Bertalanffy

Table III. - Theoretical growth parameters of *Raja* species given by different authors.

Species	Authors	L∞ (cm)	k	t_0 (year)	t_{max} (year)
R. batis	Du Buit (1977)	253.70	0.057	- 1.629	51,00
R. naevus	" "	91.64	0.085	- 0.465	27.18
R. clavata	Ryland & Ajayi (1984)	139.77	0.090	- 2.626	22.90
R. microocellata	" " "	137.00	0.086	- 3.009	23.92
R. montagui	" " "	97.80	0.156	- 1.719	13.61
R. brachyura	Holden (1972)	♂ 115,00	0.190	- 0.800	14.99
	" "	♀ 118.40	0.190	- 0.800	14.99
R. montagui	" "	♂ 68.70	0.190	- 0.560	15.23
	" "	♀ 72.80	0.180	- 0.370	16.30
R. clavata	" "	♂ 85.60	0.210	- 0.600	13.69
	" "	♀ 107,00	0.130	- 0.600	22.48
R. clavata	Brander & Palmer (1985)	105,00	0.215	- 0.450	14.40
R. miraletus	Present study	♂ 87.87	0.193	- 0.502	15,00
		♀ 91.92	0.172	- 0.247	17.20

growth parameters given by different authors for various *Raja* species. It is clear that the more longevous lived *Raja* species grow more slowly.

The breeding season in *R. miraletus* in Egyptian Mediterranean waters occurs during the period from March to September with the peak in June (Abdel-Aziz *et al.*, 1987). The incubation time for the eggs is 4-5 months (Clark, 1922). The earliest time that the young hatch is therefore August or September, with the peak in October, and the length at hatching is 8.9-11.4 cm TL. This means that the distinct length mode (8-16 cm) which appears in October surveys consists of 0-group fish. They are "young of the year", which were spawned and hatched in that calendar year. The corresponding mean length estimated from length-frequency analysis was nearly coincident with the back-calculated length at the formation of the juvenile ring derived from analysis of vertebrae sections.

The overlap in length among older age classes was caused by the decrease of growth and by the increasing variability with age class; this often made separation of size/age groups among the older fish difficult. In such cases, the fast growing younger age classes exhibit clear modes in a histogram of length-frequency which can support the interpretation of age determination (Pratt and Casey, 1983; Brander and Palmer, 1985). Tables I and II show that mean lengths corresponding to vertebral annulae I-IV were almost identical to mean length classes in the Petersen distribution. This result strongly suggests that the rings are annual, and therefore the analysis of growth increment on vertebrae is justified for age estimation in *Raja miraletus*.

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